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Received: 5 August 2021 | Accepted: 13 September 2021

DOI: 10.1002/iub.2557

SPECIAL ISSUE

Comparative analysis of the potential of the secretomes of cardiac resident stromal cells and fibroblasts

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Funding information
Brazilian National Council for Scientific and Technological Development, Funding Number/Award: 475710/2010-0; Fundação Araucaria, Funding Number/Award: 14/2009, 334/2012

Abstract

The secretome of different cell types has been applied on in vitro and in vivo assays, indicating considerable therapeutic potential. However, the choice of the ideal cell type and culture conditions for obtaining the best set of soluble factors, as well as the assays to assess specific effects, remain subjects of vigorous debate. In this study, we used mass spectrometry to characterize the secretomes of ventricle derived-cardiac resident stromal cells (vCRSC) and human dermal fibroblasts (HDFs) and evaluate them in an effort to understand the niche specificity of biological responses toward different cellular behaviors, such as cell proliferation, adhesion, migration, and differentiation. It was interesting to note that the HDF and vCRSC secretomes were both able to induce proliferation and cardiac differentiation of H9c2 cells, as well as to increase the adhesion activity of H9c2 cells and human umbilical vein endothelial cells. Analysis of the secretome composition showed that the vCRSCs derived from different donors secreted a similar set of proteins. Despite the differences, almost half of the proteins identified in conditioned medium were common to both HDF and vCRSC. Consequently, a high number of common biological processes were identified in the secretomes of the two cell types, which could help to explain the similar results observed in the in vitro assays. We show that soluble factors secreted by both HDF and vCRSC are able to promote proliferation and differentiation of cardiomyoblasts in vitro. Our study indicates the possible use of vCRSC or HDF secretomes in acellular therapies for regenerative medicine.

KEYWORDS

cardiac differentiation, cardiac resident stromal cells, conditioned medium, dermal fibroblast, secretome

Abbreviations: CM, conditioned medium; fCM, HDF-derived conditioned medium; HDFs, human dermal fibroblasts; MSC, mesenchymal stem/ stromal cells; nCM, nonconditioned medium; vCM, vCRSC-derived conditioned medium; vCRSCs, human ventricle-derived cardiac resident stromal cells.

Jhonatan Basso Lino and Anny Waloski Robert contributed equally to this work.

1 | INTRODUCTION

Cells are influenced by the microenvironment of the surrounding tissues, with dramatic changes to the cell's behavior caused by even slight changes to its milieu. Stem cell niches, defined as a subset of cells associated

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with the extracellular matrix and soluble factors, play key roles in the balance of self-renewal and cellular differentiation, participating in the cellular maintenance, preservation of phenotypic plasticity, immunological surveillance, and activation of angiogenesis.[1]

In previous work, our group isolated and characterized a heterogeneous population of human cardiac resident stromal cells (CRSCs) from two cardiac regions (right auricle and ventricle).[2,3] At that time, we showed that CRSCs' secretome was able to enhance the proliferation of mesenchymal stem cells (MSCs) and the H9c2 cardiomyoblast cell line, as well as inducing cardiac differentiation in H9c2 cells and stimulating angiogenesis in endothelial cells.[3] These results indicate that the secretome derived from a specific cell/tissue population may be a relevant source of bioactive factors of interest for acellular therapies. Questions remained, however, about whether the secretome of CRSCs would be the same regardless of differences in donor-derived isolates and whether the in vitro cell stimuli would be specific to CRSCs, or whether using another cell's secretome could obtain similar results.

To elucidate these questions, we assayed conditioned media (CMs) from ventricle-derived CRSCs (vCRSCs) isolated from three different donors as well as CMs from human dermal fibroblasts (HDFs), a cell type unrelated to cardiac tissue. We compared the potential of the CMs to induce cell proliferation, adhesion, and migration, as well as cardiac differentiation and cell recovery (after induced stress), using cell models related to the cardiac niche (cardiomyoblasts and endothelial cells). We also investigate the similarities and differences in secretome composition between different vCRSC donors and between vCRSCs and HDFs.

2 | EXPERIMENTAL PROCEDURES

2.1 | Cell culture

This study was approved by the ethics committee of the Oswaldo Cruz Foundation (CAAE number: 48374715.8.0000.5248). The vCRSCs used had been previously isolated following an established methodology.[2] Briefly, vCRSCs were isolated using an explant culture method, maintained in cell culture flasks with collagen type I coating in a medium composed of Dulbecco's MegaCell supplemented with 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acid solution, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 5 ng/ml of basic fibroblast growth factor, and 5% fetal bovine serum (FBS).

HDFs from neonatal foreskin (Lonza, catalog number CC-2509) were initially maintained in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. Before CM collection, the HDFs were adapted to the same culture conditions as vCRSCs.

The rat myoblast cell line H9c2 (ATCC, catalog number CRL-1446) was cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. Meanwhile, human umbilical vein endothelial cells (HUVEC, Lonza; catalog number C2519A) were cultured according to the manufacturer's instructions using EBM-2 complete medium. All cell types were cultivated at 37°C with 5% CO_2 and were trypsinized when the cell culture reached 80–90% of confluence.

2.2 | Sample preparation and mass spectrometry analysis of CMs

The CM was prepared following the same methodology previously described by our group.[3] CMs were obtained from vCRSCs derived from two donors (vCM1 and vCM2). For the HDFs, three independent cultures were performed to collect the CM (fCM1, fCM2, and fCM3). As a control, basal culture medium (nonconditioned medium [nCM]) was also kept. All the CMs or nCM samples were quantified by a Qubit Protein Assay Kit and stored at −80°C until use. After collecting the CMs, the samples underwent mass spectrometry (MS) analysis as previously described.[3] Briefly, 30 μg of protein was resolved in 10% SDS-PAGE and followed to in-gel tryptic digestion. The peptides were analyzed in duplicate by LC–MS/MS in a Thermo Scientific Easy-nLC 1000 system coupled to an LTQ Orbitrap XL ETD (Mass Spectrometry Facility RPT02H/Carlos Chagas Institute, Fiocruz Paraná).

We compared the new data (vCM1, vCM2, and fCMs) with a CM from a prior study (vCM3, from Reference 3) by using the number of unique peptides to filter proteins and perform a qualitative analysis. The Perseus software (version 1.6.2.1) was used to exclude proteins only identified by site, potential contaminants, and reverse identifications. We also excluded proteins that appeared in nCM with more than two unique peptides. Principal component analysis (PCA) was then performed, and vCM and fCM were analyzed separately. We filtered the unique peptide rows with values that should be greater or equal to 2 in at least one sample; we then identified the proteins that were present in at least two samples.

Functional enrichment gene ontology (GO) analysis of the proteins that were found in at least two vCM or fCM samples was performed with g:Profiler (version e96_eg43_p13_563554d)[4] using the g:SCS threshold option. The GO terms relevant for biological processes

and with a p-value ≤ 0.05 were summarized by REViGO.[5] Meanwhile, the GO terms of cellular components were plotted in a bar graph. Venn diagrams were generated in FunRich software (version 3.1.3).[6]

2.3 | H9c2 proliferation and differentiation assays

In order to evaluate whether the CMs were capable of stimulating the processes of cell proliferation and cardiac differentiation, H9c2 cells were plated at a density of approximately 0.7×10^4 cells/cm^2 and cultured for periods of 7 and 15 days with the CM or nCM stimuli. Proliferating cells were identified with anti-Ki67 antibody (Abcam, catalog number: ab15580) in association with DAPI staining. Forty-nine fields were acquired for each condition using the Operetta CLS imaging system (PerkinElmer), and the quantitative analysis was performed using Harmony software (PerkinElmer). Cardiac differentiation was evaluated by immunostaining for cardiac Troponin I (cTnI) (Santa Cruz Biotechnology, catalog number: sc-8118), and the cTnI-positive area (mm^2) was measured by an Operetta CLS imaging system and analyzed by Harmony software (PerkinElmer).

2.4 | H9c2 survival assay in the presence of oxidative stress conditions

H9c2 cells were cultured in 96-well plates (6×10^3 cells/well) for 24 hr before being exposed to two oxidative stress conditions. In this study, we used 125 μM hydrogen peroxide (H_2O_2) or 250 μM sodium arsenite ($NaAsO_2$) as stressing agents. Both were separately added to the medium and kept in contact with the cells for 2 hr. The medium was then exchanged for the nCM or CM treatments, which were maintained for approximately 18 hr. Finally, the neutral red uptake assay was performed according to a previously established methodology.[7]

2.5 | Scratch wound assay

To assess the ability of CMs to stimulate the HUVEC or H9c2 migration, we performed a scratch-wound assay, in line with prior studies.[8] Briefly, 1×10^4 cells/well (96-well plates) were maintained in culture until 100% confluence was reached. We then made a scratch in the monolayer using a 200 μl micropipette tip. The cultures were washed twice with a calcium- and magnesium-free balanced salt solution to remove cell debris. Cells were subsequently incubated with the different CMs and nCM for up to 18 hr. Using the Operetta CLS imaging system (PerkinElmer), the wells were photographed at time 0 and every 6 hr thereafter for a 24-hr period. The open area (scratch) over time was analyzed using Harmony software (PerkinElmer).

2.6 | Adhesion assay

A cell adhesion assay was performed as described by Reus et al.[3] to evaluate the adhesion capacity of HUVEC and H9c2 cells when cultured with the CMs. Briefly, 2.5×10^4 cells/well were plated in 24-well plates with CMs and nCM and were maintained at 37°C with 5% CO_2 for 10, 20, and 40 min. Once the respective period had elapsed, the cells were washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde, followed by two washes with PBS. The cells were then stained with DAPI. The number of adhered cells was recorded using the Operetta CLS imaging system (PerkinElmer) with a 20x objective, and the analysis was performed in Harmony software (PerkinElmer).

2.7 | Statistical analysis

The data were analyzed with the GraphPad Prism software, version 7.00. Differences between CM treatments were evaluated using a one-way analysis of variance (ANOVA) followed by a post hoc Tukey test. An ordinary two-way ANOVA followed by Bonferroni's multiple comparisons test was performed to compare differences in the cell proliferation rate between CMs and nCM treatments after 7 and 15 days. Bar graphs represent the means $\pm$ SD. Differences with $p < 0.05$ were considered significant.

3 | RESULTS

3.1 | CMs influence proliferation and differentiation of H9c2 cells

Initially, we used a cell line of rat cardiomyoblasts, H9c2 cells, to evaluate the specificity of the possible responses triggered by cardiac cell-derived CMs (vCM) or by HDF-derived CMs (fCM). Their potential to stimulate proliferation and cardiomyogenic differentiation was examined, as well as their ability to recover cells from stress situations.

In light of our previous results with vCM,[3] we now compared the role of vCM derived from the cells of different donors and fCM in influencing the proliferation and

differentiation of H9c2 cells after both 7 and 15 days of cell culture. We clearly observed that both vCMs and fCM could induce differentiation of H9c2 cells relative to nCM (Figure 1a), which indicates that the cardiomyogenic differentiation of H9c2 cells is not exclusively induced by secretomes derived from cells related to

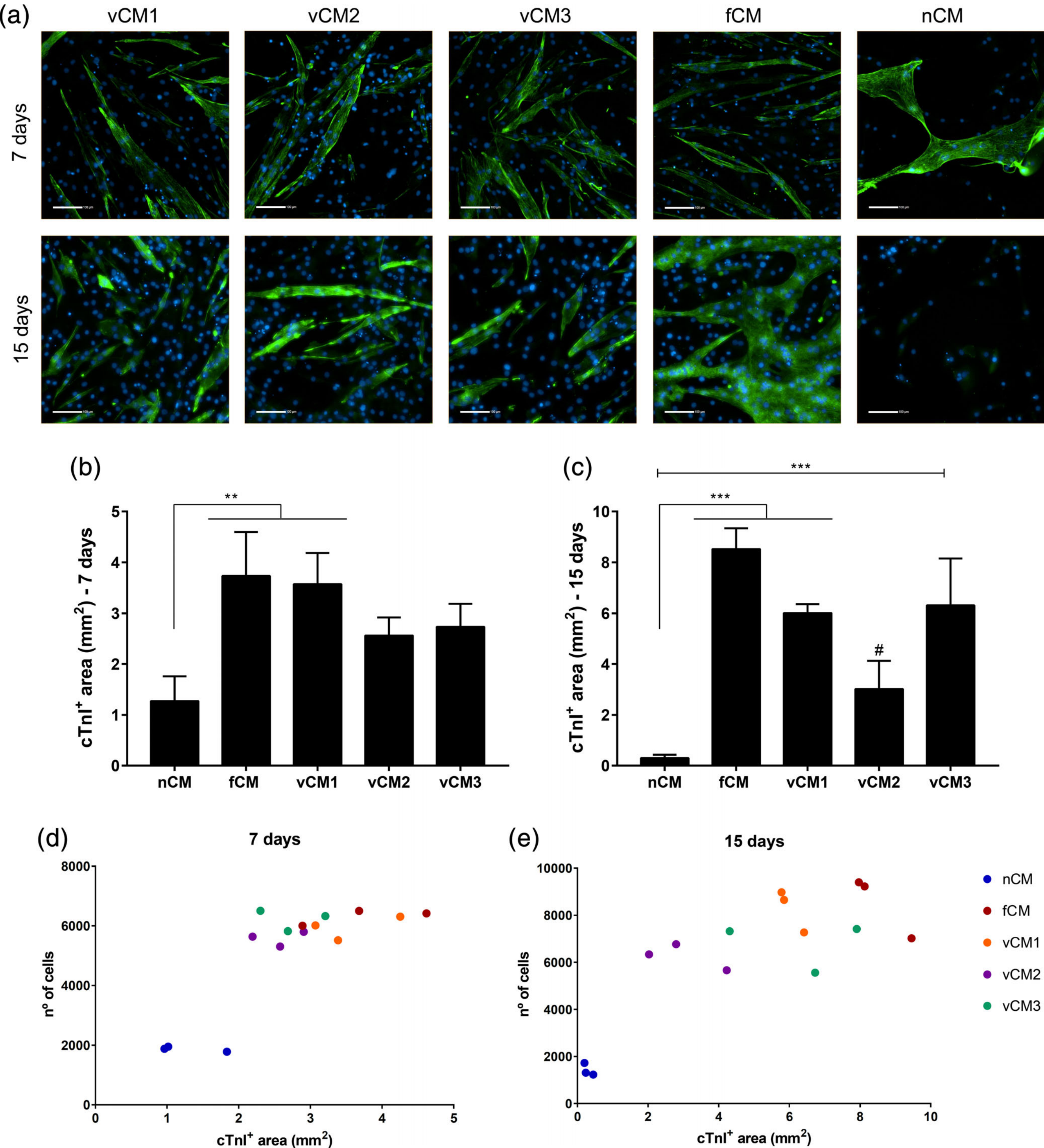

FIGURE 1 Conditioned media (CMs) can stimulate H9c2 cardiac differentiation. (a) Representative images of H9c2 cells stained with cardiac Troponin I (cTnI) after 7 (upper panel) and 15 (bottom panel) days of culture with vCRSC-derived conditioned medium (vCM), HDF-derived conditioned medium (fCM), and nonconditioned medium (nCM). Scale bar = 100 μm. Green-stained cTnI areas (in mm^2) are shown in (b) and (c) for 7 and 15 days of culture, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$, $\#p < 0.05$ in relation to fCM, vCM 1 and vCM3. Graphs (d) and (e) represent the number of nuclei per well in relation to the cTnI$^+$ area for 7 and 15 days of culture, respectively. Each colored dot symbolizes a single secretome replicate derived from independent donor cells or culture

the cardiac tissue. After 7 days, fCM and vCM-treated cells showed a greater cTnI-stained area than nCM (Figure 1b). The stained area increased even more after 15 days, with more differentiated cells in all CMs than in the nCM (Figure 1c). Long-term treatment with nCM seems to have favored the maintenance of non-differentiated H9c2 cells. Curiously, despite these differences, the use of vCM2 did not induce a higher level of H9c2 differentiation at 15 days than at 7 days and was not statistically different from nCM at any point (Figure 1c). In addition, we plotted the number of nuclei per well in relation to the $cTnI^{+}$ area, which indicated that H9c2 cells treated with CMs could simultaneously undergo elevated differentiation as well as proliferation (Figure 1d,e).

When investigating proliferation by Ki67 staining, we noticed that at 7 days, fCM had a higher percentage of Ki67-labeled cells than nCM or vCM2 and vCM3 (Figure 2a,b). However, after 15 days of treatment, fCM and vCM3 had lower proliferation rates than nCM, vCM1, and vCM2, which had significantly more $Ki67^{+}$ cells in culture (Figure 2a,c). Despite the high percentage of $Ki67^{+}$ cells in nCM after 15 days of culture (Figure 2c), nCM did not effectively stimulate proliferation since cell numbers remained the same between 7 and 15 days (Figure 2d). Furthermore, our results indicate that CMs from both HDFs and vCRSCs can stimulate the proliferation of H9c2 cells.

We also investigated the recovery after stress in H9c2 cells when treated with CMs after exposure to $NaAsO_2$ or H_2O_2. Relative cell viability levels were similar for vCM, fCM, and nCM (Supplementary Figure 1).

3.2 | vCM improves adhesion—but not migration—of H9c2 cells and HUVEC

Another important process involved in injury recovery is related to the cell migration capacity, which is an area of interest for the treatment of various diseases. We evaluated the potential of CM to promote migration of H9c2 and HUVEC using the scratch assay. Our results show that the percentage of reduction in the opened area (% closed area relative to the time of scratch) was very similar for both cell types after 6 and 18 hr under different treatments (nCM, fCM, and vCMs) (Figure 3), except for the HUVEC cultured in the presence of vCM1 for 18 hr, in which migration was greater than nCM. This indicates that neither fCM nor vCM improves the migration capacity of H9c2 and HUVEC cells relative to nCM.

There were significant differences between treatments in cell adhesion dynamics at the initial time points for both H9c2 and HUVEC, most markedly at 10 min (Figure 4). For H9c2 cells, there was an increase in the number of adhered cells at 10 and 20 min for vCM1 and vCM3 relative to the other treatments (Figure 4a–c). After 40 min, the number of adhered cells was very similar between CM treatments but significantly greater than nCM (Figure 4a,d) in nearly all cases, except fCM and vCM2. Meanwhile, the differences with HUVEC became apparent after 10 min, when a markedly higher number of cells were once again observed under the vCM1 and vCM3 treatments (Figure 4e,f). There were few differences in adhesion observed after 20 and 40 min across all CMs (Figure 4g,h), while nCM continued to induce less cell adhesion than the CMs. These results showed that the CMs were able to stimulate the adhesion process of the H9c2 and HUVEC cells.

3.3 | vCM and fCM secretome characterization

Considering that both fCM and vCM stimulated cell differentiation, proliferation, and adhesion, we investigated the secretome composition of these cells in an attempt to understand the similarities and differences between them. We compared the composition of vCM obtained from three different donors (the same ones used in the functional assays) with the fCM obtained from three technical replicates.

Initially, we confirmed the immunophenotypic profile of cell populations (Supplementary Table 1). vCRSCs were a heterogeneous population,[3,9] showing slight differences in the expression of cell surface antigens across donors, as was the case for CD90, ALPL, CD146, and CD117. HDFs showed a greater expression of CD90 and CD140b than the ventricle cells, while the expression profile was similar between both types for CD105, CD73, DDR2, and α-SMA. None of the cell types expressed significant levels of hematopoietic markers (Supplementary Table 1).

MS analysis identified between 44 and 75 proteins (with at least two unique peptides) in the different CM samples (Supplementary Table 2). Both Pearson correlation (Supplementary Figure 2a) and the Venn diagram (Figure 5a) showed that the secretomes were similar across vCM donors and fCM replicates. PCA indicates a significant difference between the composition of vCM and fCM since they were grouped separately (Figure 5b).

We considered only proteins found in at least two donors/replicates for each of the CMs to compare the composition and GO analysis of vCM and fCM. Almost half of the proteins (30 out of 55 for vCM and 58 for fCM) were shared among both CMs (Figure 5c). The common

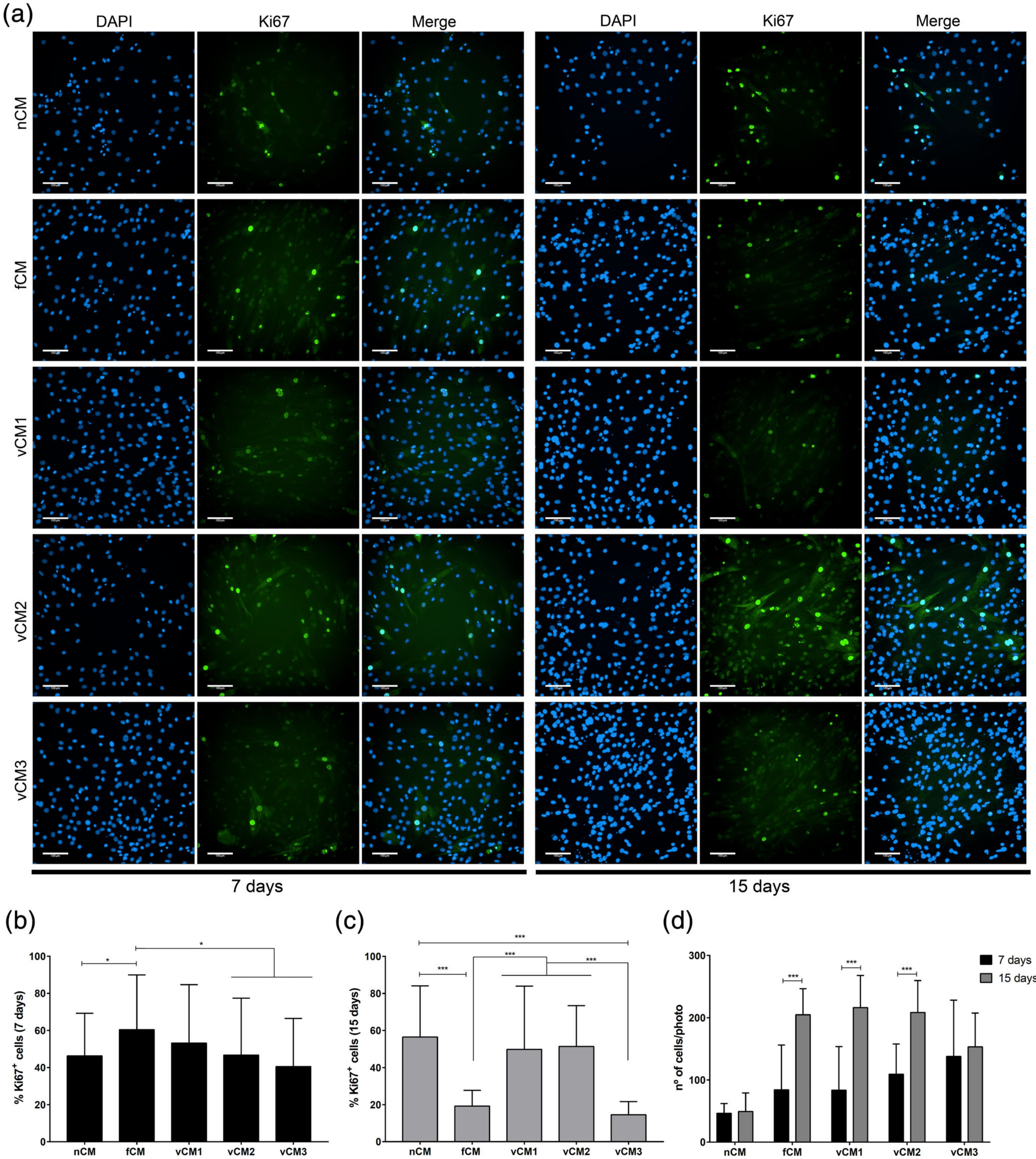

FIGURE 2 Conditioned media (CMs) can stimulate H9c2 proliferation. (a) Representative images of H9c2 cells stained with DAPI and Ki67 after 7 (left panel) and 15 (right panel) days of culture with vCRSC-derived conditioned medium (vCM), HDF-derived conditioned medium (fCM), and nonconditioned medium (nCM). Scale bar = 100 μm. Percentage of proliferating cells (%Ki67$^+$ cells) after 7 (b) and 15 (c) days of culture with different CM treatments. (d) Absolute number of cells after 7 and 15 days of culture with different CM treatments. *$p < 0.05$, ***$p < 0.001$

proteins were primarily related to ECM and included collagen types, biglycan, metalloproteinases, and others (Supplementary Table 3). However, proteins such as IL6, IGFBP7, and HSPG2, were only identified in vCM, while we found even more ECM-related proteins in fCM (e.g., PCOLCE, FBLNs, DCN, and LUM; Figure 5c).

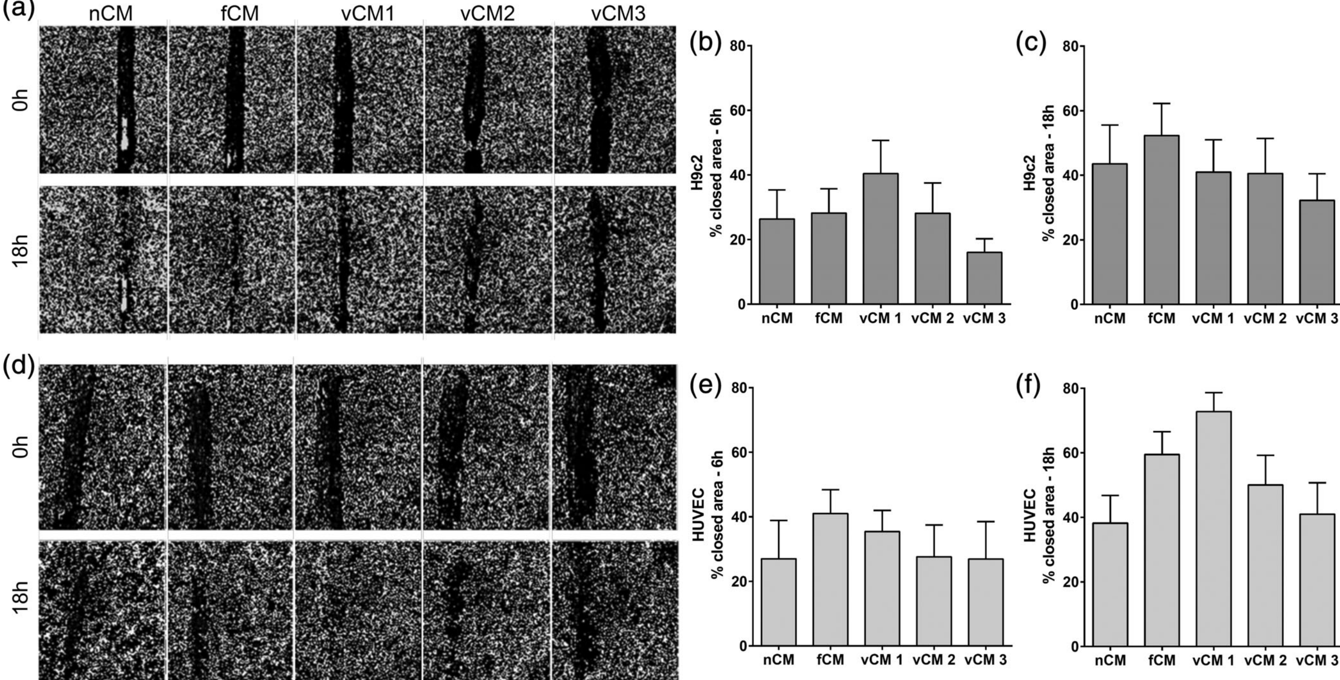

FIGURE 3 Conditioned media (CMs) do not influence the migration of H9c2 cells and human umbilical vein endothelial cells (HUVEC). (a) Representative images of H9c2 cells after 0 and 18 hr of treatment with CMs and nonconditioned medium (nCM). Graphs (b) and (c) depict the mean percentage closed area in the H9c2 scratch wound assay after 6 and 18 hr with treatments. (d) Representative images of HUVEC after 0 and 18 hr of treatment with CMs and nCM. Graphs (e) and (f) depict the mean percentage of closed area in the HUVEC scratch wound assay after 6 and 18 hr with treatments

GO analysis confirmed that CMs are enriched with cellular components related to the extracellular region, including terms from the extracellular matrix and vesicles (Figure 5d). Of the 114 terms found in fCM and the 153 terms identified in vCM, 85 are shared in both secretomes, with variations in the *p*-value (Supplementary Table 4, Supplementary Figure 2b). Interestingly, the GOs related to the 30 proteins identified in both CMs include processes like wound healing, cell adhesion, and cell migration (Figure 5e). Analysis of the proteins identified exclusively in fCM or vCM demonstrated that while fCM has some processes related to tissue development (Figure 5f), the processes identified in vCM include cell activation and immune and stress responses (Figure 5g).

4 | DISCUSSION

A growing scientific interest has emerged in understanding how the secretome of various cell types can shed light on signaling in different tissue niches, as well as its potential to emerge as a therapeutic alternative (acellular therapy) for various types of diseases. In the adult cardiac microenvironment, one area of research seeks to understand the signals that could allow the regeneration of the injured cardiac tissue and how we can stimulate it using the secretome obtained from different cell types. In this study, we showed that vCRSCs and HDFs secrete a range of proteins with the potential to stimulate different cellular responses, and, despite the different cell origins, the secretomes share many proteins and exhibit similar in vitro effects.

However, assessing the effects of the secretome in vitro is quite complex. Its composition can vary depending on the donor, tissue origin, and methodology of cell cultures (e.g., culture medium) which may lead to different results. In one example, the secretome of MSCs was isolated from different sources or donors. Although all of them were characterized as MSCs[10] with similar proteins in their secretome, significant variations were found in the concentration of individual proteins. These differences can result, for example, in a greater immunomodulatory or angiogenic potential.[11,12] However, it is not always possible to observe these behaviors in in vitro assays. Assoni et al. showed that, with a pool of CMs from MSC obtained from different donors and tissues, there was a decrease in apoptosis and an increase in migration of Duchenne muscular dystrophy myoblasts. However, the individual effects of each donor's secretome were shown to be very variable: while some CMs reduced cell apoptosis, others were not different from the control, and some even increased cell death.[13]

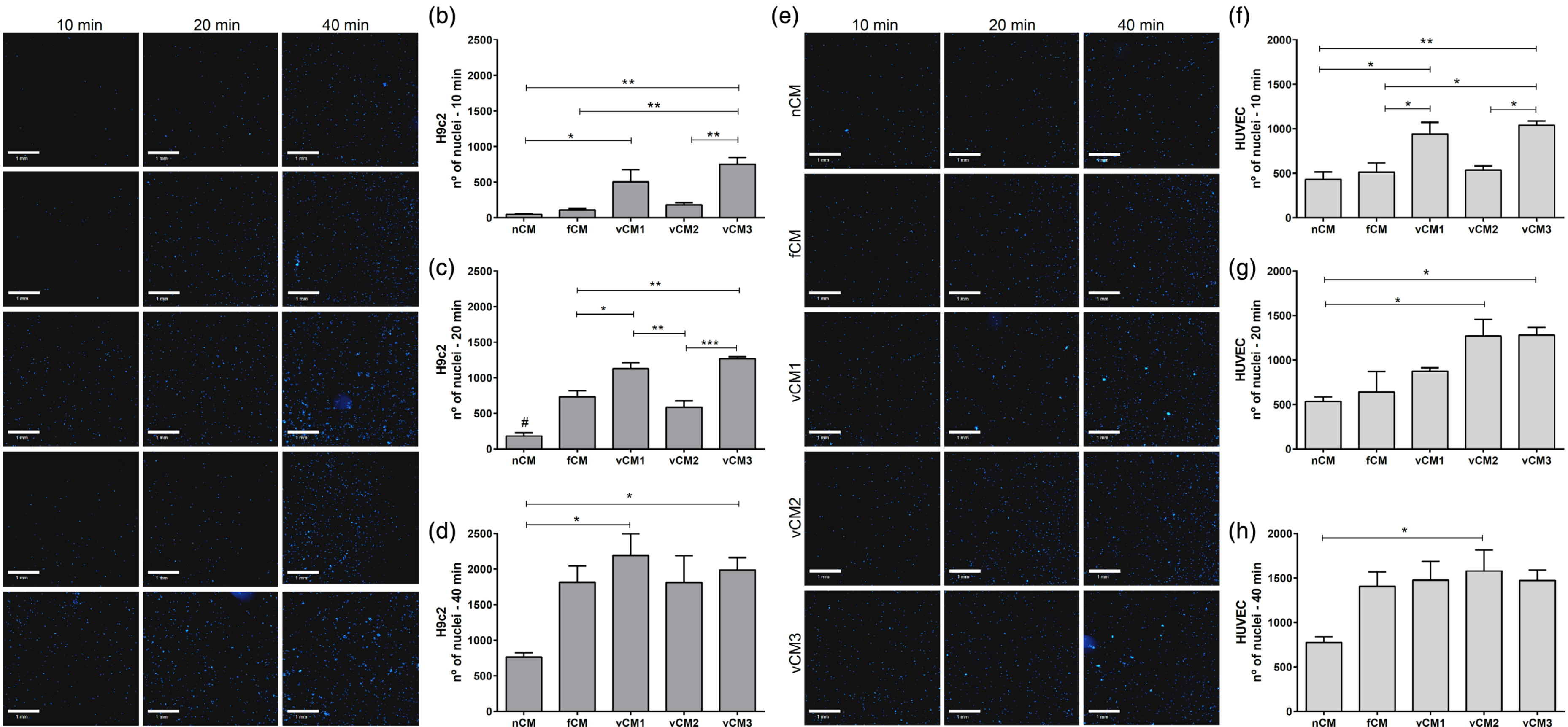

FIGURE 4 Conditioned media (CMs) can influence the adhesion of H9c2 cells and human umbilical vein endothelial cells (HUVEC). (a) Representative images of adhered H9c2 cells after 10, 20, and 40 min with different treatments. Graphs depict the number of adhered H9c2 cells after 10 (b), 20 (c), and 40 (d) min when plated in culture with CMs and nonconditioned medium (nCM). (e) Representative images of adhered HUVEC after 10, 20, and 40 min with different treatments. Number of adhered HUVEC after 10 (f), 20 (g), and 40 (h) min when plated in culture with CMs and nCM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, #$p < 0.05$ in relation to fCM and all vCMs. Scale bar = 1 mm

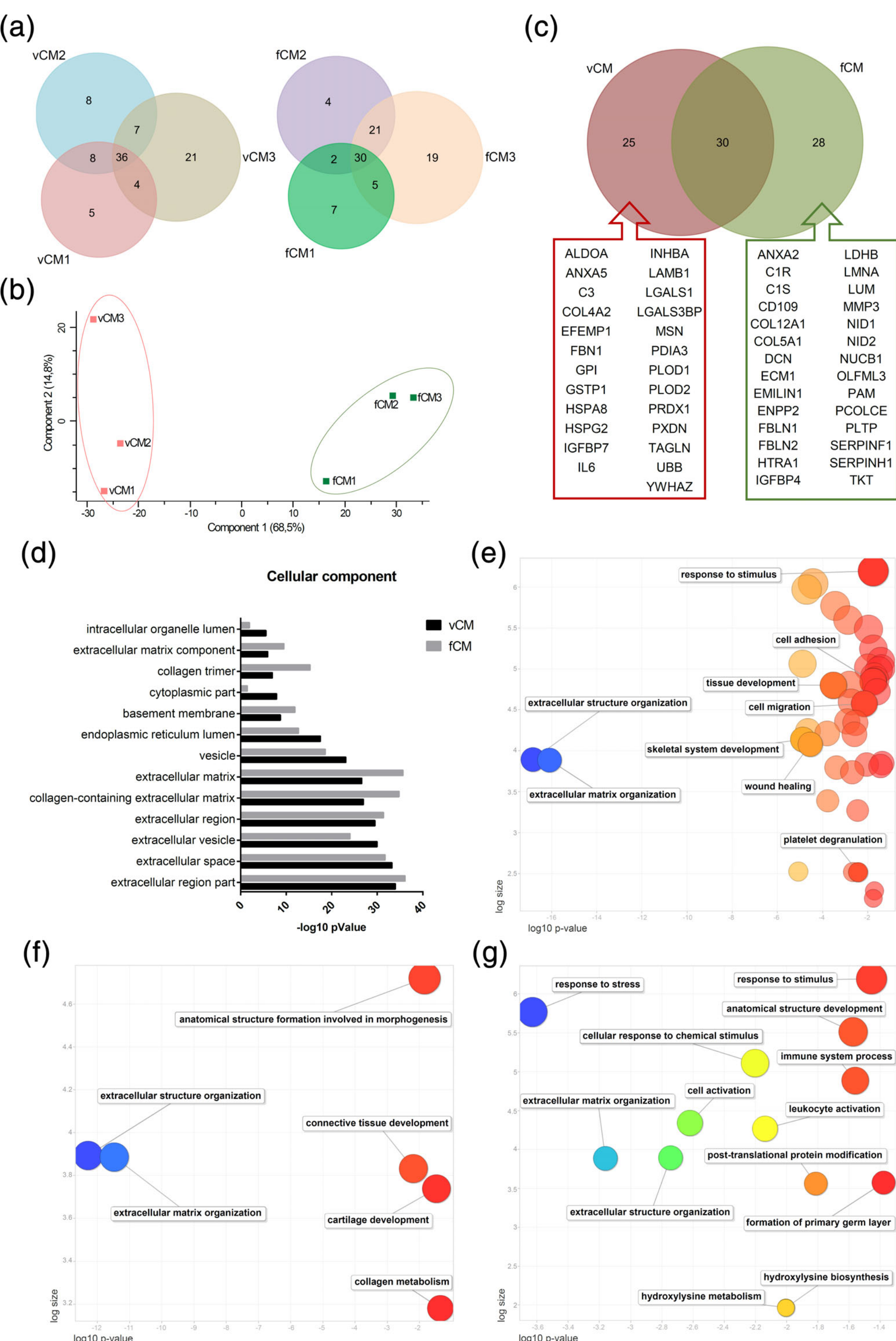

FIGURE 5 Characterization of conditioned media (CMs) obtained from ventricle-derived cardiac resident stromal cells (vCRSCs) and human dermal fibroblasts (HDFs). (a) Venn diagram representing the number of common proteins identified in vCRSC-derived conditioned medium (vCM) donors or HDF-derived conditioned medium (fCM) replicates. (b) Principal component analysis (PCA) of vCM and fCM replicates. (c) Venn diagram representing the comparison of vCM and fCM proteins. (d) Comparison of some gene ontology (GO) terms related to cellular components in vCM and fCM. REVIGO scatterplots of the GO analysis of the biological processes of proteins identified in both vCM and fCM (e), those proteins found only in fCM (f) and only in vCM (g)

In this study, we sought to verify the specificity and compare the secretome response using in vitro models with CMs derived from three vCRSC donors and dermal fibroblasts. The vCRSC and HDF secretomes induced similar effects related to proliferation, cardiac differentiation, migration, and adhesion in cultures with H9c2 cells or HUVEC. We were able to confirm our previous findings showing that vCM improved proliferation and differentiation of H9c2 cells concerning nCM.[3] Here, we demonstrated that this potential of induction does not depend on the vCRSC donor. We further observed that the cell secretome from an unrelated cardiac source (HDFs) could also stimulate these processes, indicating that our in vitro strategies demonstrate limited source specificity (at least in terms of the H9c2 results). Alternatively, these results may indicate that we have found a

new cell source from which soluble factors with the potential to assist cardiac regeneration therapy can be isolated.

We have shown that fCM and vCM from different donors were able to stimulate cardiac differentiation and proliferation of H9c2 cells, with little variation between them. In order to explore these results, we performed a proteomic characterization of the CM, which demonstrated that most of the identified proteins in the secretome profile of vCM were common to all donors (even with data generated in different runs), which supports data reproducibility. The 36 proteins identified in all three vCM included FN1, TGFB1, BGN, IL6, IGFBP7, and others. Although the cells used in this work are a heterogeneous population of cardiac cells, many of the proteins found have also been identified in the cardiac progenitor cells or cardiac fibroblast secretome, among others.[14–16]

The fCM analysis identified more than 50 proteins in at least two replicates, most of which were related to ECM. The fibroblast secretome has already been analyzed by other groups,[17,18] who found several proteins also identified in our data, such as DCN, FN1, FBLN1-2, MMP3, and others. It has also been shown that this CM, depending on cell culture conditions, has the potential to induce migration and proliferation of keratinocytes,[19,20] among other cell types, as well as promoting angiogenesis[21] and improving the maintenance of rat primary hepatocyte culture.[22]

Interestingly, when we compare the secretomes of vCRSC and HDF, despite the differences evidenced by PCA (Figure 5), we noticed many proteins that were common to both. Analysis of the GO terms related to each CM showed that both vCM and fCM might act on processes including wound healing, adhesion, angiogenesis, and others. Moreover, most of the proteins involved in these processes were the ones common to both CMs. This could help to explain, at least in part, the similar results we obtained with CMs derived from different sources (cardiac and dermal tissues).

The similarity in CM protein content may be because we use the same culture medium for both cell types. It may also be explained by the very similar immunophenotypic profile between the cell populations (as well as between the cell populations and MSCs) or because the different combinations and concentrations of proteins in the CMs, which induced the same biological process. The fact that vCRSC and HDF are likely derived from mesenchymal precursors may help explain the observation that both cell types secrete a wide range of ECM proteins. Additionally, it should be noted that the secretome characterization and comparison were made qualitatively. There may be variations in the concentration of proteins presented in each CM that could explain the differences (and similarities) in cellular responses.

It was shown that, under the experimental conditions of this study, both HDF and vCRSC secrete proteins that have the potential to stimulate important processes related to tissue regeneration. This may indicate a possible alternative source of cells for cardiac therapy, which are much easier to isolate than vCRSC. Moreover, our results indicate that, in vitro, we were unable to observe the specificity of a cell niche and that the effects of CMs seem to be pleiotropic and independent of the tissue from which the cells applied to harvest these CMs originated. However, it opens up the possibility that the factors secreted by fibroblasts can also be used in future in vivo assays. The comparison of secretome content and the similar in vitro assay results obtained here could help identify cell signals that may be causing the desired cell behaviors. Further studies are needed to better understand the effects of these CMs, using other in vitro and in vivo assays, including, for example, ones that explore the different processes highlighted between vCM and fCM, such as those related to the immune response.

5 | CONCLUSIONS

Our findings showed that the set of proteins and other molecules secreted by both vCRSCs and HDFs has the potential to stimulate cell signaling in culture, inducing proliferation and cardiac differentiation of H9c2 cells, as well as stimulating adhesion of H9c2 cells and HUVEC. Using the HDF secretome may therefore hold promise as a more practical source that remains potentially effective in acellular therapy strategies.

ACKNOWLEDGMENTS
The authors would like to thank all the staff of the Carlos Chagas Institute (FIOCRUZ/PR) for the laboratory and administrative support and the Program for Technological Development in Tools for Health-PDTIS-FIOCRUZ for the use of its facilities. We also thanks the financial support from Fundação Araucaria, the Brazilian National Council for Scientific and Technological Development and Carlos Chagas Institute (FIOCRUZ/PR). The authors are grateful to the staff of Homograft Bank (Santa Casa de Misericordia/ PUCPR) for tissue collection. The authors would also like to thank PerkinElmer for allowing the use of their Operetta high content imaging system.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest.

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How to cite this article: Lino JB, Robert AW, Stimamiglio MA, de Aguiar AM. Comparative analysis of the potential of the secretomes of cardiac resident stromal cells and fibroblasts. IUBMB Life. 2021;1–11. https://doi.org/10.1002/iub.2557